

=> d his

(FILE 'HOME' ENTERED AT 11:41:45 ON 06 JAN 2003)

FILE 'REGISTRY' ENTERED AT 11:42:03 ON 06 JAN 2003

L1 0 S MPR71292/CN  
L2 0 S MPR 71292/CN  
L3 0 S EHD2/CN  
L4 1 S ETHIDIUM HOMODIMER 2/CN

FILE 'CA' ENTERED AT 11:44:29 ON 06 JAN 2003  
S 180389-01-9/REG#

FILE 'REGISTRY' ENTERED AT 11:45:00 ON 06 JAN 2003

L5 1 S 180389-01-9/RN

FILE 'CA' ENTERED AT 11:45:00 ON 06 JAN 2003

L6 14 S L5  
L7 0 S MPR71292  
L8 0 S MPR 71292  
L9 0 S 71292  
L10 42783 S SPERM OR SPERMATOZOA OR SEMEN  
L11 58803 S VIABILITY OR MOTILITY  
L12 47707 S LIVE  
L13 105729 S L11 OR L12  
L14 23399 S CYTOMETRY OR FACS  
L15 72 S L10 AND L13 AND L14  
L16 5951 S L10 AND L11  
L17 2251562 S CONCENTRATION  
L18 26226 S FERTILITY  
L19 429150 S RELATIONSHIP  
L20 60 S L16 AND L17 AND L18 AND L19  
L21 32127 S POLYVINYL ALCOHOL

FILE 'REGISTRY' ENTERED AT 12:25:39 ON 06 JAN 2003

L22 0 S L21/CN  
L23 0 S POLYVINYL ALCOHOL/CN  
L24 27 S POLYVINYL ALCOHOL  
L25 742461 S POLYMER  
L26 124035 S COPOLYMER  
L27 742461 S L25 OR L26  
L28 9 S L24 NOT L27  
L29 1127 S VINYL ALCOHOL  
L30 42 S L29 NOT L27  
L31 1127 S VINYL ALCOHOL  
L32 742461 S POLYMER  
L33 1085 S L31 AND L32  
L34 0 FLIE CA  
L35 1 S 9002-89-5

FILE 'CA' ENTERED AT 12:31:40 ON 06 JAN 2003  
S 9002-89-5/REG#

FILE 'REGISTRY' ENTERED AT 12:31:45 ON 06 JAN 2003

L36 1 S 9002-89-5/RN

FILE 'CA' ENTERED AT 12:31:45 ON 06 JAN 2003

L37 47415 S L36

L38 30 S L37 AND L10

FILE 'BIOSIS' ENTERED AT 13:27:28 ON 06 JAN 2003

L39 61617 S SPERM OR SPERATOZOA OR SEMEN  
L40 51112 S VIABILITY  
L41 996255 S CORRELATION OR RELATIONSHIP OR CORRELATED OR CORRELATES  
L42 57711 S FERTILITY OR NONRETURN  
L43 85 S L42 AND L41 AND L40 AND L39

FILE 'USPATFULL' ENTERED AT 13:56:20 ON 06 JAN 2003

L44 267 S L39 AND L40 AND L41 AND L42  
L45 588875 S CONCENTRATION OR (CELL NUMBER)  
L46 256 S L44 AND L45  
L47 113011 S FLUORESCEN? OR (SYBR 14) OR (PROPIDIUM IODIDE) OR ETHIDIUM  
L48 188 S L47 AND L46  
L49 6687 S FACS OR (FLOW CYTOMER)  
L50 52 S L48 AND L49

FILE 'BIOSIS' ENTERED AT 14:45:30 ON 06 JAN 2003

L51 2890 S MULTIPAROUS  
L52 41 S L39 AND L51  
L53 71112 S SPERMATOZOA OR SEMEN OR SPERM  
L54 48 S L53 AND L51  
L55 7 S L54 NOT L52  
L56 1882 S L53 AND L40  
L57 6150 S LITTER SIZE  
L58 8916 S L57 OR L51  
L59 25 S L58 AND L56  
L60 3612 S FERTILITY AND (LITTER SIZE) OR MULTIPAROUS  
L61 210 S L60 AND L53  
L62 17 S L40 AND L61

FILE 'MEDLINE' ENTERED AT 15:06:19 ON 06 JAN 2003

L63 18 S L62

FILE 'WPIDS' ENTERED AT 15:07:17 ON 06 JAN 2003

L64 0 S L62  
L65 23 S L53 AND FERTILITY AND VIABILITY

=> log hold

|  |            |         |
|--|------------|---------|
| COST IN U.S. DOLLARS                       | SINCE FILE | TOTAL   |
|  | ENTRY      | SESSION |
| FULL ESTIMATED COST                        | 14.00      | 475.64  |
| DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS) | SINCE FILE | TOTAL   |
|  | ENTRY      | SESSION |
| CA SUBSCRIBER PRICE                        | 0.00       | -21.08  |

SESSION WILL BE HELD FOR 60 MINUTES

STN INTERNATIONAL SESSION SUSPENDED AT 15:09:48 ON 06 JAN 2003

Connection closed by remote host

=> d his

(FILE 'HOME' ENTERED AT 11:41:45 ON 06 JAN 2003)

FILE 'REGISTRY' ENTERED AT 11:42:03 ON 06 JAN 2003

L1 0 S MPR71292/CN  
L2 0 S MPR 71292/CN  
L3 0 S EHD2/CN  
L4 1 S ETHIDIUM HOMODIMER 2/CN

FILE 'CA' ENTERED AT 11:44:29 ON 06 JAN 2003  
S 180389-01-9/REG#

FILE 'REGISTRY' ENTERED AT 11:45:00 ON 06 JAN 2003

L5 1 S 180389-01-9/RN

FILE 'CA' ENTERED AT 11:45:00 ON 06 JAN 2003

L6 14 S L5  
L7 0 S MPR71292  
L8 0 S MPR 71292  
L9 0 S 71292  
L10 42783 S SPERM OR SPERMATOZOA OR SEMEN  
L11 58803 S VIABILITY OR MOTILITY  
L12 47707 S LIVE  
L13 105729 S L11 OR L12  
L14 23399 S CYTOMETRY OR FACS  
L15 72 S L10 AND L13 AND L14  
L16 5951 S L10 AND L11  
L17 2251562 S CONCENTRATION  
L18 26226 S FERTILITY  
L19 429150 S RELATIONSHIP  
L20 60 S L16 AND L17 AND L18 AND L19  
L21 32127 S POLYVINYL ALCOHOL

FILE 'REGISTRY' ENTERED AT 12:25:39 ON 06 JAN 2003

L22 0 S L21/CN  
L23 0 S POLYVINYL ALCOHOL/CN  
L24 27 S POLYVINYL ALCOHOL  
L25 742461 S POLYMER  
L26 124035 S COPOLYMER  
L27 742461 S L25 OR L26  
L28 9 S L24 NOT L27  
L29 1127 S VINYL ALCOHOL  
L30 42 S L29 NOT L27  
L31 1127 S VINYL ALCOHOL  
L32 742461 S POLYMER  
L33 1085 S L31 AND L32  
L34 0 FLIE CA  
L35 1 S 9002-89-5

FILE 'CA' ENTERED AT 12:31:40 ON 06 JAN 2003  
S 9002-89-5/REG#

FILE 'REGISTRY' ENTERED AT 12:31:45 ON 06 JAN 2003

L36 1 S 9002-89-5/RN

FILE 'CA' ENTERED AT 12:31:45 ON 06 JAN 2003

L37 47415 S L36  
L38 30 S L37 AND L10

FILE 'BIOSIS' ENTERED AT 13:27:28 ON 06 JAN 2003

L39 61617 S SPERM OR SPERATOZOA OR SEMEN  
L40 51112 S VIABILITY  
L41 996255 S CORRELATION OR RELATIONSHIP OR CORRELATED OR CORRELATES  
L42 57711 S FERTILITY OR NONRETURN  
L43 85 S L42 AND L41 AND L40 AND L39

FILE 'USPATFULL' ENTERED AT 13:56:20 ON 06 JAN 2003

L44 267 S L39 AND L40 AND L41 AND L42  
L45 588875 S CONCENTRATION OR (CELL NUMBER)  
L46 256 S L44 AND L45  
L47 113011 S FLUORESCEN? OR (SYBR 14) OR (PROPIDIUM IODIDE) OR ETHIDIUM  
L48 188 S L47 AND L46  
L49 6687 S FACS OR (FLOW CYTOMER)  
L50 52 S L48 AND L49

FILE 'BIOSIS' ENTERED AT 14:45:30 ON 06 JAN 2003

L51 2890 S MULTIPAROUS  
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FILE 'MEDLINE' ENTERED AT 15:06:19 ON 06 JAN 2003

L63 18 S L62

FILE 'WPIDS' ENTERED AT 15:07:17 ON 06 JAN 2003

L64 0 S L62  
L65 23 S L53 AND FERTILITY AND VIABILITY

FILE 'USPATFULL, USPAT2' ENTERED AT 15:29:49 ON 06 JAN 2003

L66 15559 FILE USPATFULL  
L67 173 FILE USPAT2  
TOTAL FOR ALL FILES  
L68 15732 S SPERM OR SPERMATOZOA OR SEMEN  
L69 402017 FILE USPATFULL  
L70 4870 FILE USPAT2  
TOTAL FOR ALL FILES  
L71 406887 S MICROPARTICLES OR PARTICLES  
L72 5849 FILE USPATFULL  
L73 67 FILE USPAT2  
TOTAL FOR ALL FILES  
L74 5916 S L71 AND L68  
L75 8609 FILE USPATFULL  
L76 106 FILE USPAT2  
TOTAL FOR ALL FILES  
L77 8715 S (FLOW CYTOMETER) OR FACS  
L78 1323 FILE USPATFULL  
L79 11 FILE USPAT2  
TOTAL FOR ALL FILES  
L80 1334 S L77 AND L74  
L81 244 FILE USPATFULL

L82            2 FILE USPAT2  
           TOTAL FOR ALL FILES  
 L83            246 S L68 (P) L71  
 L84            29 FILE USPATFULL  
 L85            1 FILE USPAT2  
           TOTAL FOR ALL FILES  
 L86            30 S L77 AND L83

          FILE 'WPIDS' ENTERED AT 15:41:17 ON 06 JAN 2003  
 L87            3471 S SPERM OR SPERMATOZOA OR SEMEN  
 L88            254044 S MICROPARTICLES OR PARTICLES  
 L89            83 S L87 AND L88

=> log hold

|  |            |         |
|--|------------|---------|
| COST IN U.S. DOLLARS                       | SINCE FILE | TOTAL   |
|  | ENTRY      | SESSION |
| FULL ESTIMATED COST                        | 51.06      | 580.73  |
| DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS) | SINCE FILE | TOTAL   |
|  | ENTRY      | SESSION |
| CA SUBSCRIBER PRICE                        | 0.00       | -21.08  |

SESSION WILL BE HELD FOR 60 MINUTES  
 STN INTERNATIONAL SESSION SUSPENDED AT 15:48:07 ON 06 JAN 2003

=> d his

(FILE 'HOME' ENTERED AT 11:41:45 ON 06 JAN 2003)

FILE 'REGISTRY' ENTERED AT 11:42:03 ON 06 JAN 2003

L1 0 S MPR71292/CN  
L2 0 S MPR 71292/CN  
L3 0 S EHD2/CN  
L4 1 S ETHIDIUM HOMODIMER 2/CN

FILE 'CA' ENTERED AT 11:44:29 ON 06 JAN 2003  
S 180389-01-9/REG#

FILE 'REGISTRY' ENTERED AT 11:45:00 ON 06 JAN 2003

L5 1 S 180389-01-9/RN

FILE 'CA' ENTERED AT 11:45:00 ON 06 JAN 2003

L6 14 S L5  
L7 0 S MPR71292  
L8 0 S MPR 71292  
L9 0 S 71292  
L10 42783 S SPERM OR SPERMATOZOA OR SEMEN  
L11 58803 S VIABILITY OR MOTILITY  
L12 47707 S LIVE  
L13 105729 S L11 OR L12  
L14 23399 S CYTOMETRY OR FACS  
L15 72 S L10 AND L13 AND L14  
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L18 26226 S FERTILITY  
L19 429150 S RELATIONSHIP  
L20 60 S L16 AND L17 AND L18 AND L19  
L21 32127 S POLYVINYL ALCOHOL

FILE 'REGISTRY' ENTERED AT 12:25:39 ON 06 JAN 2003

L22 0 S L21/CN  
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L24 27 S POLYVINYL ALCOHOL  
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L31 1127 S VINYL ALCOHOL  
L32 742461 S POLYMER  
L33 1085 S L31 AND L32  
L34 0 FLIE CA  
L35 1 S 9002-89-5

FILE 'CA' ENTERED AT 12:31:40 ON 06 JAN 2003  
S 9002-89-5/REG#

FILE 'REGISTRY' ENTERED AT 12:31:45 ON 06 JAN 2003

L36 1 S 9002-89-5/RN

FILE 'CA' ENTERED AT 12:31:45 ON 06 JAN 2003

L37 47415 S L36  
L38 30 S L37 AND L10

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 L50 52 S L48 AND L49

=> log hold

|                      |            |         |
|----------------------|------------|---------|
| COST IN U.S. DOLLARS | SINCE FILE | TOTAL   |
|                      | ENTRY      | SESSION |
| FULL ESTIMATED COST  | 7.62       | 397.46  |

|  |            |         |
|--|------------|---------|
| DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS) | SINCE FILE | TOTAL   |
|  | ENTRY      | SESSION |
| CA SUBSCRIBER PRICE                        | 0.00       | -21.08  |

SESSION WILL BE HELD FOR 60 MINUTES  
 STN INTERNATIONAL SESSION SUSPENDED AT 13:59:38 ON 06 JAN 2003

=> d his

(FILE 'HOME' ENTERED AT 11:41:45 ON 06 JAN 2003)

FILE 'REGISTRY' ENTERED AT 11:42:03 ON 06 JAN 2003

L1 0 S MPR71292/CN  
L2 0 S MPR 71292/CN  
L3 0 S EHD2/CN  
L4 1 S ETHIDIUM HOMODIMER 2/CN

FILE 'CA' ENTERED AT 11:44:29 ON 06 JAN 2003  
S 180389-01-9/REG#

FILE 'REGISTRY' ENTERED AT 11:45:00 ON 06 JAN 2003

L5 1 S 180389-01-9/RN

FILE 'CA' ENTERED AT 11:45:00 ON 06 JAN 2003

L6 14 S L5  
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L8 0 S MPR 71292  
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L32 742461 S POLYMER  
L33 1085 S L31 AND L32  
L34 0 FLIE CA  
L35 1 S 9002-89-5

FILE 'CA' ENTERED AT 12:31:40 ON 06 JAN 2003  
S 9002-89-5/REG#

FILE 'REGISTRY' ENTERED AT 12:31:45 ON 06 JAN 2003

L36 1 S 9002-89-5/RN

FILE 'CA' ENTERED AT 12:31:45 ON 06 JAN 2003

L37 47415 S L36  
L38 30 S L37 AND L10



=> log hold

COST IN U.S. DOLLARS

SINCE FILE

TOTAL

ENTRY

SESSION

FULL ESTIMATED COST

29.64

292.72

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)

SINCE FILE

TOTAL

ENTRY

SESSION

CA SUBSCRIBER PRICE

-4.34

-21.08

SESSION WILL BE HELD FOR 60 MINUTES

STN INTERNATIONAL SESSION SUSPENDED AT 12:36:23 ON 06 JAN 2003

L33 ANSWER 1085 OF 1085 REGISTRY COPYRIGHT 2003 ACS

RN 9002-89-5 REGISTRY

CN **Ethenol, homopolymer (9CI)** (CA INDEX NAME)

OTHER CA INDEX NAMES:

CN **Vinyl alcohol, polymers (8CI)**

OTHER NAMES:

CN Acroflex 1

CN Acroflex 2

CN AH 17

CN AH 22

CN AH 26

CN Aibon AU 7002FL

CN Airvol 103

CN Airvol 107

CN Airvol 107SF

CN Airvol 125

CN Airvol 125SF

CN Airvol 165

CN Airvol 165SF

CN Airvol 166

CN Airvol 21-205

CN Airvol 21-25

CN Airvol 24-203

CN Airvol 321LA

CN Airvol 325

CN Airvol 325SF

CN Airvol 350

CN Airvol 350SF

CN Airvol 425

CN Airvol 502

CN Airvol 53

CN Airvol 710

CN Airvol 803

CN Airvol V 205

CN Airvol WS 42

CN AL 6

CN **AL 6 (polymer)**

CN Alcotex 17F-H

CN Alcotex 72.5L

CN Alcotex 75L

CN Alcotex 99/10

CN Alvyl

CN AQ 2117

CN Aquafilm L 330

CN Aquareserve GP 02

CN Aquareserve GP 48

CN Aracet APV

CN Aracet APV 120-88

CN Aracet APV 50-92

CN Aracet APV 50/88

CN **Atactic poly(vinyl alcohol)**

CN AX 300SN

CN B 17

CN B 20F

CN Bansuta PX 25

CN BF 24

CN **C 10 (vinyl polymer)**

CN **C 20 (vinyl polymer)**

CN **K 16 (polymer)**

CN M 1000 (vinyl polymer)  
CN NP 25 (vinyl polymer)  
CN P 610 (vinyl polymer)  
CN Poly(vinyl alcohol)  
CN PV 03 (vinyl alcohol polymer)  
CN TTF (polymer)  
CN Vinyl alcohol homopolymer  
CN Vinyl alcohol polymer  
CN Warcopolymer A 20

ADDITIONAL NAMES NOT AVAILABLE IN THIS FORMAT - Use FCN, FIDE, or ALL for  
DISPLAY

DR 9014-14-6, 9050-53-7, 9066-05-1, 162261-31-6, 53241-16-0, 58740-50-4,  
25038-51-1, 98002-48-3, 106442-33-5, 61584-38-1, 73298-53-0, 75923-48-7,  
147827-36-9, 151439-02-0, 153569-70-1, 152987-51-4, 155421-52-6,  
39320-29-1, 353276-42-3, 372077-13-9

MF (C2 H4 O)x

CI PMS, COM

PCT Polyvinyl

LC STN Files: ADISNEWS, AGRICOLA, ANABSTR, AQUIRE, BIOBUSINESS, BIOSIS,  
BIOTECHNO, CA, CABA, CANCERLIT, CAPLUS, CASREACT, CBNB, CEN, CHEMLIST,  
CHEMSAFE, CIN, CSCHM, CSNB, DDFU, DETHERM\*, DIOGENES, DRUGU, EMBASE,  
ENCOMPLIT, ENCOMPLIT2, ENCOMPPAT, ENCOMPPAT2, HSDB\*, IFICDB, IFIPAT,  
IFIUDB, IPA, MEDLINE, MRCK\*, MSDS-OHS, NIOSHTIC, PDLCOM\*, PHARMASEARCH,  
PIRA, PLASPEC\*, PROMT, RTECS\*, TOXCENTER, TULSA, USAN, USPAT2,  
USPATFULL, VTB

(\*File contains numerically searchable property data)

Other Sources: DSL\*\*, TSCA\*\*

(\*\*Enter CHEMLIST File for up-to-date regulatory information)

CM 1

CRN 557-75-5

CMF C2 H4 O

$\text{H}_2\text{C}=\text{CH}-\text{OH}$

47368 REFERENCES IN FILE CA (1962 TO DATE)

3610 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA

47408 REFERENCES IN FILE CAPLUS (1962 TO DATE)

=>

L38 ANSWER 14 OF 30 CA COPYRIGHT 2003 ACS

AN 121:4426 CA

TI In vitro fertilization of bovine oocytes in a chemically defined, protein-free medium varying the bicarbonate concentration

AU Tajik, Parviz; Wang, Wei Hua; Okuda, Kiyoshi; Niwa, Koji

CS Fac. Agric., Okayama Univ., Okayama, 700, Japan

SO Biology of Reproduction (1994), 50(6), 1231-7

CODEN: BIREBV; ISSN: 0006-3363

DT Journal

LA English

AB Bovine cumulus-enclosed oocytes were matured in culture, freed from cumulus cells, and inseminated with frozen-thawed **spermatozoa** in a chem. defined protein-free medium contg. 5 mM caffeine and 10  $\mu$ g/mL heparin. No penetration of oocytes was obsd. in the medium without polyvinylalc. (PVA); but when the medium was supplemented with 0.1-5 mg/mL

PVA, penetration rates (9-16%) significantly increased. **Sperm** motility was also stimulated during incubation for 2 h in the presence of PVA. In the medium with 1 mg/mL PVA, a high penetration rate (24 of 62=39%) was obsd. at a **sperm** concn. of 10.times.10<sup>6</sup> cells/mL.

When the bicarbonate concn. was changed in the fertilization medium contg.

1 mg/mL PVA and 10.times.10<sup>6</sup> **spermatozoa**/mL, a high penetration rate (47 of 67=70%) and a high proportion (44 of 47=94%) of oocytes in which male and female pronuclei had developed were obtained at 46 mM NaHCO<sub>3</sub>. However, the penetration rate (58-95%), the incidence of pronuclear formation (64-96%), and the proportion of polyspermy (9-21%) varied according to the animal (five different bulls).

**Spermatozoa** obtained from two bulls started to penetrate oocytes 5 h after insemination in the presence of 46 mM NaHCO<sub>3</sub>. This is the first report indicating that induction of capacitation of bull **spermatozoa** and penetration of oocytes matured in culture are possible in a chem. defined, protein-free medium.

L38 ANSWER 13 OF 30 CA COPYRIGHT 2003 ACS

AN 123:140269 CA

TI Functional analysis using chlortetracycline fluorescence and in vitro fertilization of frozen-thawed ejaculated boar **spermatozoa** incubated in a protein-free chemically defined medium

AU Wang, W. H.; Abeydeera, L. R.; Fraser, L. R.; Niwa, K.

CS Fac. Agric., Okayama Univ., Okayama, 700, Japan

SO Journal of Reproduction and Fertility (1995), 104(2), 305-13

CODEN: JRPFA4; ISSN: 0022-4251

PB Journals of Reproduction and Fertility Ltd.

DT Journal

LA English

AB Cumulus-enclosed pig oocytes were matured in vitro, freed from cumulus cells, and inseminated with frozen-thawed ejaculated **spermatozoa** in a chem. defined protein-free medium contg. 37.0 mmol NaHCO<sub>3</sub> L-1 and 5 mmol caffeine L-1. When the medium was supplemented with 1 mg polyvinylalc. (PVA) mL-1, more penetrated oocytes were obsd. 14 h after insemination with 7-12 .times. 106 cells mL-1 than with 4-5 .times. 106 cells mL-1 and the incidence of polyspermy reflected the **sperm** concn. used. Varying the NaHCO<sub>3</sub> concn. but maintaining the **sperm** concn. at 8 .times. 106 cells mL-1 resulted in significantly more oocytes being penetrated in media contg. 45.83-50.25 than 37.0-41.42 mmol NaHCO<sub>3</sub> L-1; there were no significant differences in the incidence of either

male

pronuclear formation or polyspermy. In medium contg. 45.83 mmol NaHCO<sub>3</sub> L-1, the inclusion of PVA at 0-5 mg mL-1 had no effect on proportions of penetrated oocytes, male pronuclear formation or polyspermy. However, when **spermatozoa** from three different boars were evaluated, the penetration and male pronuclear formation rates were highly variable, unlike the incidence of polyspermy. Penetration of cumulus-free oocytes was first detected at 6 h. When **spermatozoa** were incubated for 6 h in the absence of oocytes, motility, but not vitality, decreased whether or not PVA was included in the medium. Chlortetracycline (CTC) fluorescence anal. of the capacitation state indicated a rapid decline in the proportion of live uncapacitated, acrosome-intact cells and a rapid rise in the proportion of live capacitated, acrosome-reacted cells during the first hour. Smaller changes in the distribution of CTC patterns occurred during the later stages, suggesting that the rapidly responding cells were non-fertilizing, owing to damage by freeze-thawing, and that the fertilizing **spermatozoa** were drawn from the remaining pool of cells which underwent capacitation more slowly. This is the first report indicating that capacitation of frozen-thawed ejaculated boar **spermatozoa** and penetration of oocytes matured in culture are possible in a chem. defined, protein-free medium.

EH 72

L6 ANSWER 14 OF 14 CA COPYRIGHT 2003 ACS

AN 125:162751 CA

TI Fluorescent viability assay using cyclic-substituted unsymmetrical cyanine

dyes

IN Millard, Paul J.; Roth, Bruce L.; Yue, Stephen T.; Haugland, Richard P.

PA Molecular Probes, Inc., USA

SO U.S., 26 pp., Cont. of U. S. 5,436,134.

CODEN: USXXAM

DT Patent

LA English

FAN.CNT 8

|      | PATENT NO.                                | KIND | DATE     | APPLICATION NO. | DATE     |
|------|---|------|----------|-----------------|----------|
| PI   | US 5534416                                | A    | 19960709 | US 1993-148847  | 19931108 |
|      | US 5436134                                | A    | 19950725 | US 1993-90890   | 19930712 |
|      | US 5545535                                | A    | 19960813 | US 1993-146328  | 19931101 |
|      | CA 2133765                                | AA   | 19941027 | CA 1994-2133765 | 19940413 |
|      | EP 675924                                 | A1   | 19951011 | EP 1994-914173  | 19940413 |
|      | EP 675924                                 | B1   | 20011212 |                 |          |
|      | R: AT, BE, CH, DE, ES, FR, GB, IT, LI, NL |      |          |                 |          |
|      | AT 210703                                 | E    | 20011215 | AT 1994-914173  | 19940413 |
|      | ES 2166777                                | T3   | 20020501 | ES 1994-914173  | 19940413 |
|      | JP 07196930                               | A2   | 19950801 | JP 1994-159824  | 19940712 |
| PRAI | US 1993-47683                             | B2   | 19930413 |                 |          |
|      | US 1993-90890                             | A1   | 19930712 |                 |          |
|      | US 1993-146328                            | A2   | 19931101 |                 |          |
|      | US 1993-148847                            | A    | 19931108 |                 |          |
|      | WO 1994-US4127                            | W    | 19940413 |                 |          |

OS MARPAT 125:162751

AB The invention relates to a method of analyzing the viability of a sample of cells using an aq. soln. comprising two fluorescent dyes. Dye I has the formula I where R2 is C1-6 alkyl; Z- is a biol. compatible counterion;

X is O, S, Se, or NR15, where R15 is H or C1-6 alkyl; or CR16R17, where R16 and R17, which may be the same or different, are independently H or C1-6 alkyl, or the carbons of R16 and R17 taken in combination complete a 5- or 6-membered satd. ring; and the benzazolium is optionally further substituted; n = 0, 1, or 2; Y is CR3:CR4; p and m = 0 or 1, such that p

+ m = 1; R5 is a C1-6 alkyl, C1-6 alkenyl, C1-6 polyalkenyl, C1-6 alkynyl, or C1-6 polyalkynyl group; or R5 is an OMEGA; R3, R4, R6 and R7, which

may be the same or different, are independently H; or a C1-6 alkyl, C1-6 alkenyl, C1-6 polyalkenyl, C1-6 alkynyl or C1-6 polyalkynyl group; or halogen; or OR8, SR8, (NR8R9), where R8 and R9, which may be the same or different, are independently H; or alkyl groups having 1-6 carbons; or

1-2 substituted or unsubstituted alicyclic, heteroalicyclic, arom., or heteroarom. rings, contg. 1-4 heteroatoms, wherein the heteroatoms are O, N, or S. R8 and R9 taken in combination are (CH2)2L(CH2)2 where L = O, NR10, CH2 or a single bond where R10 is H or an alkyl group having 1-6 carbons; or OSO2R19 where R19 is C1-6 alkyl, or C1-6 perfluoroalkyl, or aryl; or an OMEGA; or R6 and R7, taken in combination are (CH2)v where v

= 3 or 4, or R6 and R7 form a fused arom. ring that is optionally further substituted; such that at least one of R3, R4, R5, R6 and R7, or a substituent on the arom. ring formed by R6 and R7, is an OMEGA; where OMEGA is a cyclic substituent that is attached by a single bond.

Fluorescent Dye II selectively stains either viable or nonviable cells with a detectable fluorescent response that is different from the fluorescent response of Dye I. The stained cells are illuminated at a suitable absorption wavelength, and the fluorescent response is detected to distinguish viable and nonviable cells based on the fluorescent response.

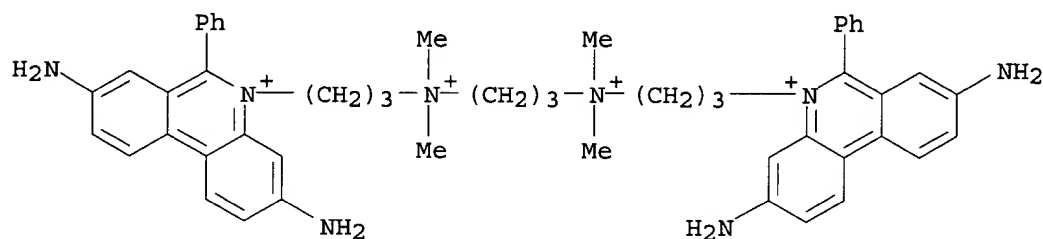
=>

L6 ANSWER 14 OF 14 CA COPYRIGHT 2003 ACS  
IT 596-09-8, Fluorescein diacetate 1239-45-8, Ethidium bromide 3348-03-6  
3546-21-2, Ethidium 24147-36-2, Thiazole orange 25535-16-4, Propidium  
iodide 36015-30-2, Propidium 38483-26-0 61926-22-5, Ethidium  
homodimer 63783-82-4, Ethidium monoazide 105284-17-1 124412-00-6  
127770-45-0 139626-15-6, Tetramethylrhodamine ethyl ester 163831-68-3  
169454-17-5 180388-99-2 180389-00-8 **180389-01-9**  
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)  
(fluorescent cell viability assay using cyclic-substituted unsym.  
cyanine dyes)



> d

L4 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2003 ACS  
RN 180389-01-9 REGISTRY  
CN Phenanthridinium, 5,5'-[1,3-propanediylbis[(dimethyliminio)-3,1-propanediyl]]bis[3,8-diamino-6-phenyl-, tetraiodide (9CI) (CA INDEX NAME)  
OTHER NAMES:  
CN Ethidium homodimer 2  
MF C51 H60 N8 . 4 I  
SR CA  
LC STN Files: CA, CAPLUS, CHEMCATS, TOXCENTER, USPAT2, USPATFULL



● 4 I<sup>-</sup>

14 REFERENCES IN FILE CA (1962 TO DATE)  
14 REFERENCES IN FILE CAPLUS (1962 TO DATE)

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=> d bib ab 6

L6 ANSWER 6 OF 14 CA COPYRIGHT 2003 ACS  
AN 135:207869 CA  
TI Method and reagent for counting sperm by flow cytometry  
IN Matsumoto, Teruya; Okada, Hiroshi; Hamaguchi, Yukio  
PA Sysmex Co., Ltd., Japan  
SO Jpn. Kokai Tokkyo Koho, 6 pp.  
CODEN: JKXXAF  
DT Patent  
LA Japanese  
FAN.CNT 1

|      | PATENT NO.    | KIND | DATE     | APPLICATION NO. | DATE     |
|------|---------------|------|----------|-----------------|----------|
| PI   | JP 2001242168 | A2   | 20010907 | JP 2000-52953   | 20000229 |
|      | US 2001024806 | A1   | 20010927 | US 2001-790368  | 20010222 |
|      | US 6472168    | B2   | 20021029 |                 |          |
| PRAI | JP 2000-52953 | A    | 20000229 |                 |          |

OS MARPAT 135:207869

AB A method and a reagent are provided for accurately counting sperm even in a seminal fluid sample contg. impurities. A seminal fluid sample is treated with an aq. soln. contg. a cationic surfactant (e.g., quaternary ammonium salt, pyridinium salt), and stained with a nucleic acid-staining dye (e.g., ethidium bromide, propidium iodide, N-methyl-4-(1-pyrene)vinylpropidium iodide, TOTO-1, TOTO-3, YOYO-1, YOYO-3, BOBO-1, BOBO-3, ethidium homodimer-1 (Ethd-1), ethidium homodimer-2 (Ethd-2), POPO-1, POPO-3, BO-PRO-1, YO-PRO-1, TO-PRO-1). Then, the sperm in the sample is counted by flow cytometry.

L15 ANSWER 58 OF 72 CA COPYRIGHT 2003 ACS

AN 120:157733 CA

TI Flow cytometric analysis for reproductive biology

AU Spano, Marcello; Evenson, Donald P.

CS Div. Mol. Bio. Biophys. Bioelectron., CRE Casaccia, Rome, 00060, Italy

SO Biology of the Cell (1993), 78(1-2), 53-62

CODEN: BCELDF; ISSN: 0248-4900

DT Journal; General Review

LA English

AB A review with 127 refs. Flow cytometric studies of spermatogenesis have been advanced by the need for: (i) rapid, sensitive, objective and multiparameter measurements of reproductive effects due to environmental, occupational, and therapeutic exposure to toxicants; and (ii) assessment of fertility potential of human and animal **sperm**. As a consequence, various flow cytometric techniques are already available to identify germ cell subpopulations undergoing both proliferative and maturative processes in normal and perturbed conditions. Significant improvements have been introduced to investigate the spermatogenic

complex

differentiation pathway and the apparent uniformity of mature **sperm**. Flow **cytometry** (FCM) has been applied to the measurement of both testis and **sperm** cells in a variety of species, including man. End points considered in toxicol. studies are: altered testicular germ cell ratios, DNA and RNA content, increase of the coeff. of variation, induction of diploid elongated spermatids and

diploid

**sperm**, altered nuclear morphol., **sperm** cell **viability**, mitochondrial function and **sperm** chromatin structure. Precise DNA content measurements allow accurate anal. to det. the proportion of X- and Y-chromosome bearing **sperm** and sorting of these subpopulations for gender preselection. FCM technol. has

reached

a maturation level that allows its inclusion in the list of available and routine methods for reproductive studies in human and animal populations.

L43 ANSWER 78 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
AN 1985:392373 BIOSIS  
DN BA80:62365  
TI FLUOROMETRY OF POULTRY **SEMEN** ITS APPLICATION IN THE  
DETERMINATION OF **VIABILITY** ENZYME LEAKAGE AND **FERTILITY**

AU BILGILI S F; RENDEN J A; SEXTON K J  
CS POULTRY SCI. DEP., ALA. AGRIC. EXP. STN., AUBURN UNIV., ALA. 36849.  
SO POULT SCI, (1985) 64 (6), 1227-1230.  
CODEN: POSCAL. ISSN: 0032-5791.

FS BA; OLD

LA English

AB The accuracy of fluorometry for estimating percentages of dead [Single  
Comb White Leghorn] chicken spermatozoa was investigated by comparing

this technique with the eosin-nigrosin differential staining procedure and  
with

glutamic oxaloacetic transaminase (GOT) concentration in seminal plasma.  
The **relationship** between percent dead **sperm** measured  
by fluorometry and **fertility** was also examined. The  
**correlation** coefficient of percentage of dead spermatozoa  
determined by fluorometry with eosin-nigrosin counts was highly  
significant ( $r = 0.99$ ;  $P < 0.001$ ). Similarly, the **correlation**  
coefficient of GOT activity with percentage of dead spermatozoa was 0.99  
( $P < 0.001$ ). Percent **fertility**, fertile egg production and  
duration of **fertility** were negatively **correlated** with  
percent dead spermatozoa, -0.55, -0.51 and -0.44 ( $P < 0.001$ ),  
respectively.

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Via FAX 3/26/85

L43 ANSWER 73 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
 AN 1987:416963 BIOSIS  
 DN BA84:83625  
 TI USE OF LINEAR **SEMEN** QUALITY SCORE FOR CLASSIFICATION AND  
 DECISION MAKING IN EVALUATION OF INDIVIDUAL EJACULATES OF HOLSTEIN  
 BULLS.  
 AU CHANDLER J E; ADKINSON R W; SMITH J W; SAXTON A M  
 CS DEP. DAIRY SCI., LOUISIANA AGRIC. EXP. STN., LA. STATE UNIV. AGRIC.  
 CENT.,  
 BATON ROUGE 70893.  
 SO J DAIRY SCI, (1987) 70 (5), 1036-1044.  
 CODEN: JDSCAE. ISSN: 0022-0302.  
 FS BA; OLD  
 LA English  
 AB Four hundred seven ejaculates from 15 Holstein bulls collected from  
 December 1984 to June 1985 were evaluated postthaw for **viability**  
 characteristics (percent progressive motility at 0 h and after 3 h at  
 37.degree.C incubation, percent intact acrosomal membrane after 3 h at  
 37.degree.C incubation) and abnormal morphological characteristics  
 [percent head (primary), midpiece, and tail (secondary) abnormalities].  
 Weighting coefficients for combining **viability** and abnormality  
 characteristics were generated from between-bull and within-bull  
 variance  
 and covariance matrices. Two hundred ninety-eight additional ejaculates  
 collected from July 1985 to February 1986 were added. Linear quality  
 scores for 705 ejaculates (24 bulls) were the sum of the product of each  
 quality characteristic and weighting coefficients. Univariate analysis  
 yielded significant bull effects for **viability** and abnormality  
 characteristics and linear quality score. Significant **correlations**  
 existed between all seminal quality characteristics except primary and  
 secondary abnormalities. A t test with preassigned critical value was  
 used  
 to evaluate each ejaculate to determine rejection from the population.  
 Percent of ejaculates rejected was lower when linear quality score was  
 used than when five independent tests were used. Use of linear quality  
 score to critique **semen** based on each ejaculate's innate quality  
 could compensate for the loss of bull **fertility** estimates from  
 declining number of technical-based AI programs.

L43 ANSWER 41 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
 AN 1998:41735 BIOSIS  
 DN PREV199800041735  
 TI Effect of cryopreservation on bovine **sperm viability**  
 as determined by dual DNA staining.  
 AU Garner, D. L. (1); Thomas, C. A.; Allen, C. H.; Senger, P. L.; Sasser, R.  
 G.  
 CS (1) Sch. Vet. Med., Univ. Nevada, Reno, NV 89557 USA  
 SO Reproduction in Domestic Animals, (Dec., 1997) Vol. 32, No. 6, pp.  
 279-283.  
 ISSN: 0936-6768.  
 DT Article  
 LA English  
 SL English; German  
 AB The proportions of living and damaged spermatozoa in samples of 24  
 h-stored and cryopreserved spermatozoa from six bulls were determined  
 using dual fluorescent staining of DNA and flow cytometry. In the 24  
 h-stored samples, the mean proportion of living spermatozoa was 60.3 +-  
 6.3%, while the mean proportion after cryopreservation was 40.3 +- 4.0%.  
 Significant differences ( $p < 0.01$ ) were found among these bulls in the  
 proportion of living spermatozoa as determined by staining the  
**sperm** nucleic acids before and after cryopreservation using the  
 combination of SYBR-14 and propidium iodide (PI). In addition, the  
 proportion of spermatozoa staining with SYBR-14/PI were determined in  
 samples from five bulls where **fertility** had been determined. The  
**fertility** levels of **semen** from these bulls as determined  
 by pregnancy-specific protein B, ranking from high to low, were 68.0,  
 64.7, 63.6, 60.5 and 57.1%, whereas the mean proportion of living  
 spermatozoa were 32.5, 28.2, 26.8, 14.0 and 34.4%, respectively. The  
 proportions of spermatozoa stained with SYBR-14 were not  
**correlated** with the **fertility** of the cryopreserved  
 samples from these five bulls. These results demonstrated that dual DNA  
 staining of spermatozoa can be used as an indicator of the ability of a  
 bull's spermatozoa to successfully undergo cryopreservation, but that the  
 singular as

3 ANSWER 29 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
AN 2000:322886 BIOSIS  
DN PREV200000322886  
TI Use of a **sperm** analyzer for evaluating broiler breeder males. 2.  
Selection of young broiler breeder roosters for the **sperm**  
quality index increases fertile egg production.  
AU Parker, H. M.; Yeatman, J. B.; Schultz, C. D.; Zumwalt, C. D.; McDaniel,  
C. D. (1)  
CS (1) Poultry Science Department, Mississippi State University, Mississippi  
State, MS, 39762 USA  
SO Poultry Science, (May, 2000) Vol. 79, No. 5, pp. 771-777. print.  
ISSN: 0032-5791.  
DT Article  
LA English  
SL English  
AB Previous research has shown that the **sperm** quality index (SQI)  
of rooster **semen** is indicative of overall **semen**  
quality. The objectives of the present experiments were to determine the  
**correlation** of the SQI with **semen** characteristics and  
**fertility** and to determine if selection of young males for the SQI  
would improve **fertility**. In Experiment 1 **semen** was  
collected from 35 Peterson males and was analyzed individually for  
**sperm** concentration and **viability**. To determine  
**fertility**, 100  $\mu$ L of diluted **semen** was inseminated into  
10 hens for each rooster. Positive **correlations** of the SQI with  
total and live **sperm** concentrations as well as **fertility**  
were found. A negative **correlation** of the SQI with the  
percentage of dead **sperm** was observed. In Experiment 2, four  
**semen** samples were collected at 2- to 3-d intervals from each of  
142, 27-wk-old Peterson roosters to determine their SQI. Males were then  
allocated to six treatment groups based on their average SQI readings as  
follows: 0 to 150, 151 to 200, 201 to 250, 251 to 300, 301 to 350, and  
>350. For each SQI group, **semen** was collected weekly for 8 wk,  
pooled, and used at a rate of 50  $\mu$ L/hen to inseminate 40 hens. The  
percentage of fertilized eggs increased linearly across the SQI groups,  
from a minimum of 65% for the 0 to 150 SQI group to a maximum of 98% for  
the >350 SQI group. The SQI groups of 301 to 350 and >350 produced the  
slowest decline in **fertility** over days postinsemination.  
Therefore, selection of males for the SQI at an early age appears to  
improve flock **fertility**.

L43 ANSWER 60 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
 AN 1993:294150 BIOSIS  
 DN PREV199396012375  
 TI Interrelationships among fluorometric analyses of spermatozoal function, classical **semen** quality parameters and the **fertility** of frozen-thawed bovine spermatozoa.  
 AU Ericsson, S. A. (1); Garner, D. L.; Thomas, C. A.; Downing, T. W.; Marshall, C. E.  
 CS (1) Box C-110-, Range/Anim. Sci., Sul Ross State Univ., Alpine TX 79832  
 SO Theriogenology, (1993) Vol. 39, No. 5, pp. 1009-1024.  
 ISSN: 0093-691X.  
 DT Article  
 LA English  
 AB Cryopreserved spermatozoa from 8 bulls were used to examine the interrelationships among flow cytometric spermatozoal quality assessments and classical **semen** quality parameters and **nonreturn** rate estimates of **fertility**. The integrity of the **sperm** cell membrane and the functional capacity of the mitochondria were quantified by flow cytometry after concurrent staining with carboxydimethylfluorescein diacetate (CDMFDA), propidium iodide (PI), and rhodamine 123 (R123). For each sample a total of 10,000 stained spermatozoa were simultaneously quantified for the intensity of their green and red fluorescence. Three straws from each bull were each examined initially and following incubation at 37 degree C for 3 hours to assess the rate of senescence. The proportion of spermatozoa retaining membrane integrity and having functional mitochondria, as determined by CDMFDA and R123 staining, were compared with classical **semen** quality assessments (**sperm** motility, acrosomal status, cellular and head morphology, presence of vacuoles/craters and cytoplasmic droplets) and with **fertility** (**nonreturn** to estrus rates). For individual ejaculates **nonreturn** rates, the range was from 61.8 to 78.8%, whereas the cumulative rates of several ejaculates for each bull ranged from 71.3 to 83.5%. The proportion of spermatozoa with functional membranes and mitochondria were positively **correlated** with the percentage of spermatozoa with normal morphology ( $r = 0.82$ ;  $P = 0.01$ ) and motility after 4 hours of incubation ( $r = 0.78$ ;  $P = 0.02$ ), but not with the estimates of **fertility**. The actual number of spermatozoa per straw staining with CDMFDA and R123 after 4 hours of incubation at 37 degree C was correlated with the percentage of spermatozoa with normal morphology ( $r = 0.73$ ;  $P = 0.04$ ). Multiple regression equations indicated that combinations of **semen** quality measurements could be useful in estimating fertilizing potential.



L43 ANSWER 72 OF 85 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
 AN 1988:203934 BIOSIS  
 DN BA85:105280  
 TI FLUOROMETRY OF POULTRY **SEMEN** INFLUENCE OF DILUTION AND STORAGE  
 ON CHICKEN SPERMATOZOAL **VIABILITY** AND **FERTILITY**.  
 AU BILGILI S F; SEXTON K J; RENDEN J A  
 CS POULT. SCI. DEP., ALA. AGRIC. EXP. STN., AUBURN UNIV., ALA. 36849.  
 SO POULT SCI, (1987) 66 (12), 2032-2035.  
 CODEN: POSCAL. ISSN: 0032-5791.  
 FS BA; OLD  
 LA English  
 AB Two experiments were conducted to measure the effects of **semen**  
 dilution and storage time (0, 1, 2, 3, 4, 24, and 48 h) at 22 C on  
 spermatozoal **viability** (i.e., membrane permeability to ethidium  
 bromide) and to determine the **relationship** between concentration  
 of viable spermatozoa inseminated (25, 50, 100, and 200 .times. 10<sup>6</sup>) and  
**fertility**. In Experiment 1, percentages of dead spermatozoa  
 remained relatively constant during the 4-h postcollection period but  
 increased significantly ( $P < .05$ ) at 24 and 48 h. **Sperm**  
**viability** after 48 h was significantly higher in diluted  
**semen** than in undiluted **semen**. Percent (PF) and duration  
 of **fertility** (DF) from undiluted **semen** significantly  
 declined during the 4-h postcollection period compared with  
**fertility** of diluted **semen**. In Experiment 2, both PF and  
 DF improved as the concentration of viable spermatozoa increased.  
**Fertility** was not significantly improved by inseminating more than  
 100 .times. 10<sup>6</sup> viable spermatozoa. The fertilizing capacity of chicken  
 spermatozoa from undiluted **semen** was affected during storage  
 before membrane permeability to ethidium bromide was altered.

L20 ANSWER 18 OF 60 CA COPYRIGHT 2003 ACS  
AN 133:333436 CA  
TI Seminal quality correlates with mitochondrial functionality  
AU Ruiz-Pesini, E.; Lapena, A. C.; Diez, C.; Alvarez, E.; Enriquez, J. A.;  
Lopez-Perez, M. J.  
CS Departamento de Bioquimica, Biologia Molecular y Celular, Universidad de  
Zaragoza, Zaragoza, 50013, Spain  
SO Clinica Chimica Acta (2000), 300(1-2), 97-105  
CODEN: CCATAR; ISSN: 0009-8981  
PB Elsevier Science Ireland Ltd.  
DT Journal  
LA English  
AB Oligozoospermia is an important manifestation of male subfertility and  
very little attention has been paid to study a possible  
**relationship** between the total no. of ejaculated  
**spermatozoa** and mitochondrial functionality. In this work we  
report a direct correlation between spectrophotometrically measured  
mitochondrial enzyme activities (citrate synthase and respiratory complex  
I, II, I+III, II+III and IV) and seminogram parameters (**sperm**  
**motility**, vitality and cell **conc.**). In addn., total  
ejaculated **spermatozoa** correlate much better with the  
nuclear-encoded citrate synthase and complex II than with the  
mitochondrial-encoded complex I, III and IV activities. Furthermore,  
total no. of **spermatozoa** has a significant but neg. correlation  
with the ratios of complex I, complex III and complex IV to complex II  
(and citrate synthase). These ratios are significantly higher in aged  
subjects emphasizing the physiol. relevance of this observation. These  
results suggest that the simultaneous increase of the no. of ejaculated  
**spermatozoa** and the mitochondrial enrichment of citrate synthase  
and complex II are both parallel responses to the same regulatory events.  
RE.CNT 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

8-27-00

L15 ANSWER 13 OF 72 CA COPYRIGHT 2003 ACS  
AN 134:190219 CA  
TI Fluorescent probes and flow **cytometry** to assess rat  
**sperm** integrity and mitochondrial function  
AU Gravance, Curtis G.; Garner, Duane L.; Miller, Marion G.; Berger, Trish  
CS Department of Environmental Toxicology, University of California, Davis,  
CA, 95616, USA  
SO Reproductive Toxicology (2001), 15(1), 5-10  
CODEN: REPTED; ISSN: 0890-6238  
PB Elsevier Science Inc.  
DT Journal  
LA English  
AB Fluorescent assessment of cellular integrity and mitochondrial function  
by

flow **cytometry** can provide a rapid and precise means of detg.  
the functional status of large nos. of **spermatozoa**. In the  
present study, rat **sperm viability** was assessed with  
SYBR-14 and PI and **sperm** mitochondria were differentially  
labeled with JC-1. **Sperm** samples of variable **viability**  
were prepd. using varying proportions of fresh and frozen  
**spermatozoa**. SYBR-14 stained **sperm** correlated well with  
expected **sperm viability** ( $r = 0.98$ ). Motile  
**sperm** stained with JC-1 appeared orange in the midpiece indicating  
a high mitochondrial membrane potential whereas immotile **sperm**  
with a low membrane potential stained green. The percentage of  
**spermatozoa** staining orange was highly correlated ( $r = 0.99$ ) with  
expected **sperm viability**. Flow **cytometry**  
using specific fluorescent probes is a useful technique for detecting  
changes in rat **sperm** plasma membrane integrity and mitochondrial  
function in large nos. of **spermatozoa**.

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L20 ANSWER 22 OF 60 CA COPYRIGHT 2003 ACS

AN 133:28127 CA

TI Assessment of **sperm** characteristics post-thaw and response to calcium ionophore in relation to **fertility** in Swedish dairy AI bulls

AU Januskauskas, A.; Johannisson, A.; Soderquist, L.; Rodriguez-Martinez, H.  
CS Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Centre for Reproductive Biology, Swedish University of Agricultural Sciences (SLU), Uppsala, SE-750 07, Swed.

SO Theriogenology (2000), 53(4), 859-875

CODEN: THGNBO; ISSN: 0093-691X

PB Elsevier Science Inc.

DT Journal

LA English

AB The present study examd. the **relationship** between bull **sperm** characteristics post-thawing, after swim-up, and after challenge to calcium ionophore in relation to **fertility** (56-d nonreturn rates) after artificial insemination (AI). **Spermatozoa** from 25 **semen** batches derived from 15 Swedish Red and White AI bulls were evaluated with regard to post-thaw **motility**, membrane integrity, and migration through a swim-up procedure. The swim-up sepd. **spermatozoa** were assessed in terms of **sperm concn.**, **viability** and capacitation status as well as their response to exogenous calcium ionophore (A23187). Acrosome reactions were evaluated by fluorescence microscopy and flow cytometry. **Sperm motility** and **viability** post-thawing were significantly correlated with **fertility**. For the swim-up sepd. **semen**, significant correlations to nonreturn rates were found for **concn.**, **viability**, no. of viable **spermatozoa** and **sperm** capacitation status (Pattern F and Pattern B). The only parameter significantly correlated to **fertility** after the ionophore challenge was the percentage of acrosome-reacted **spermatozoa** with remaining equatorial fluorescence, as assessed by fluorescence microscopy but not by flow cytometry. The regression anal. showed that combining the results of **sperm** membrane integrity assessment post-thawing with those of capacitation status after swim-up provided the best prediction of **fertility**. The accuracy of prediction did not improve when these parameters were combined with the percentage of **spermatozoa** in which the acrosome reaction was induced by ionophore challenge.

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